

CLAIMS

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1. An oligonucleotide comprising at least one concatenation coding for a polypeptide with formula $(P-K)_n$, where:
 - n is a whole number of 2 or more;
 - P represents a proline amino acid residue;
 - K represents a lysine amino acid residue;
 - the symbol “-” represents a bond between the two amino acid residues, in particular a peptide type bond, the n (P-K) units also being bonded together by such bonds, for example peptide type bonds.
 2. An oligonucleotide according to claim 1, comprising a concatenation coding for a polypeptide with formula $(P-K)_n$ where n is a whole number of 3 or more, and preferably n is equal to 4, 5, 6, 7, 8, 9, 10 or 15.
 3. An oligonucleotide according to claim 1 or claim 2, comprising a concatenation coding for a polypeptide with formula $(P-K)_n$, in which the sequence of n (P-K) units is interrupted by one or more amino acid residues other than P or K residues.
 4. An oligonucleotide according to any one of claims 1 to 3, characterized in that the concatenation coding for the polypeptide comprising the n (P-K) units is completed at its 5' end and/or at its 3' end by one or more codons coding, for example, for at least one lysine residue at the N-terminal extremity of the formed polypeptide.
 5. An oligonucleotide according to claim 4, characterized in that it codes for a polypeptide with formula $(P-K)_4$, formula $K-(P-K)_4$, or with formula $2K(P-K)_4$.
 6. A recombinant nucleotide sequence comprising a concatenation of nucleotides coding for a plant protein reserve, characterized in that it further comprises an oligonucleotide according to any one of claims 1 to 5, inserted at one site of the nucleotide concatenation selected such that:
 - expression of the nucleotide sequence in a particular plant cell enables a modified protein reserve to be produced which is localised in that cell in

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	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87														

- the modified protein reserve coded by the recombinant nucleotide sequence is immunologically recognised by antibodies produced against the corresponding normal protein reserve.

14. A nucleotide sequence according to claim 13, characterized in that the nucleotide concatenation coding for the maize γ -zein it contains has the sequence shown in Figure 9.

26. A lysine-enriched modified maize γ -zein, characterized in that its amino acid sequence is modified by at least one polypeptide with formula $(P-K)_n$ or with formula $2K(P-K)_n$, where:
- n is a whole number of 2 or more;
 - P represents a proline amino acid residue;
 - K represents a lysine amino acid residue;
 - the symbol “-” represents a bond between the two amino acid residues, in particular a peptide type bond, the n $(P-K)$ units being bonded together by bonds, in particular peptide type bonds, said polypeptide having formula $(P-K)_n$ or $K-(P-K)_n$ being substituted for a sequence naturally present in the normal maize γ -zein or being inserted with deletion of one or more amino acids of the amino acid sequence for normal maize γ -zein, or being added to the normal γ -zein amino acid sequence, the insertion site for the polypeptide being selected such that:
 - when the modified lysine-rich γ -zein is produced in a host cell, in particular in a plant cell, it is localised in identical or similar manner to the normal maize γ -zein which would be produced under the same conditions in the same host cell; and/or
 - the modified maize γ -zein is recognised by antibodies directed against the normal maize γ -zein.
27. A modified maize γ -zein according to claim 26, characterized in that it is the protein P20 γ Z or the protein H30 γ Z or the protein H45 γ Z.
28. A recombinant host cell, characterized in that it comprises a nucleotide sequence according to any one of claims 1 to 17.
29. A host cell according to claim 28, characterized in that it is a bacterium, for example E. coli or Agrobacterium tumefaciens.
30. A host cell according to claim 28, characterized in that it is a plant cell.
31. A host cell according to claim 30, characterized in that it is a plant seed cell.

15. A nucleotide sequence according to claim 7 or claim 8, characterized in that the coding nucleotide concatenation it comprises codes for a protein reserve of a plant selected from the following: soya, sunflower, tobacco, wheat, oats, alfalfa, rice, oilseed rape, sorghum, and Arabidopsis thaliana.
- 5 16. A nucleotide sequence according to claim 13 or claim 14, characterized in that the oligonucleotide is inserted in place of or following the Pro-X domain or in the Pro-X domain naturally present in the maize γ -zein.
17. A recombinant nucleotide sequence, characterized in that it comprises a nucleotide sequence according to any one of claims 1 to 16 under the control of an expression promoter.
- 10 18. A recombinant nucleotide sequence according to claim 17, characterized in that the promoter is a specific promoter for a given cell tissue, for example a promoter which is specific for expression in grains, and/or in the leaves of plants.
- 15 19. A nucleotide sequence according to claim 17, characterized in that the expression promoter is that of maize γ -zein.
20. A nucleotide sequence according to claim 17, characterized in that the expression promoter is the promoter CaMV35S.
21. A nucleotide sequence according to any one of claims 16 to 20, characterized in that it codes for one of the polypeptides P20 γ Z or H45 γ Z with the sequences shown in Figures 11 and 10 respectively.
- 20 22. A cloning and/or expression vector, characterized in that it comprises, at a site which is not essential for replication, a nucleotide sequence in accordance with any one of claims 1 to 20.
- 25 23. A cloning and/or expression vector, characterized in that it is one of plasmids pP20 γ Z (CNCM N° I-1640), pH30 γ Z or pH45 γ Z (CNCM N° I-1639).
24. A polypeptide coded by a sequence according to any one of claims 1 to 17.
25. A lysine-enriched modified maize γ -zein, characterized in that it is coded by a nucleotide sequence according to claim 13 or claim 14.

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32. A host cell according to claim 31, characterized in that it is a cell from maize seed endosperm.
33. A host cell according to claim 32, characterized in that it contains a nucleotide sequence according to claim 13, integrated in its genome in a stable manner.
- 5 34. A host cell according to claim 32, characterized in that it produces a lysine-enriched modified maize γ -zein according to claim 26 or claim 27.
35. A host cell according to claim 30, characterized in that it is a soya, sunflower, tobacco, wheat, oats, alfalfa, rice, oilseed rape, sorghum or Arabidopsis cell.
- 10 36. Seeds producing a polypeptide according to any one of claims 24 to 27.
37. A plant producing a polypeptide according to any one of claims 24 to 27.
38. A plant according to claim 37, characterized in that it is a maize plant.
39. Seeds obtained from plants according to claim 37 or claim 38.
- 15 40. A method of producing plants or seeds expressing a modified protein reserve, characterized in that it comprises the steps of:
- a) transforming a plant cell with a nucleotide sequence according to any one of claims 6 to 17, or a vector according to claim 22 or claim 23, under conditions enabling the modified protein reserve coded by the nucleotide sequence to be expressed in a stable and functional manner;
- 20 b) regenerating plants from the plant cell transformed in step a), to obtain plants expressing the modified protein reserve;
- c) if necessary, obtaining seeds from the modified plants obtained in step b).
- 25 41. A method according to claim 40, characterized in that the plant is maize and the protein reserve is γ -zein.

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